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(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: THOMPSON, Richard, Craig [US/US]; 763 North County Road 900 West, Frankfort, IN 46041 (US).

(74) Agents: PAGE, Kathleen, R., S. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(54) Title: UREA AND THIOUREA DERIVATIVES OF GLYCOPEPTIDES

(57) Abstract

The present invention is directed to N^{LEU}–carbamoyl and thiocarbamoyl derivatives of A82846B and N^{DISACC} variations thereof. These derivatives are useful as antibacterials and also as starting materials from which further antibacterial compounds are prepared.

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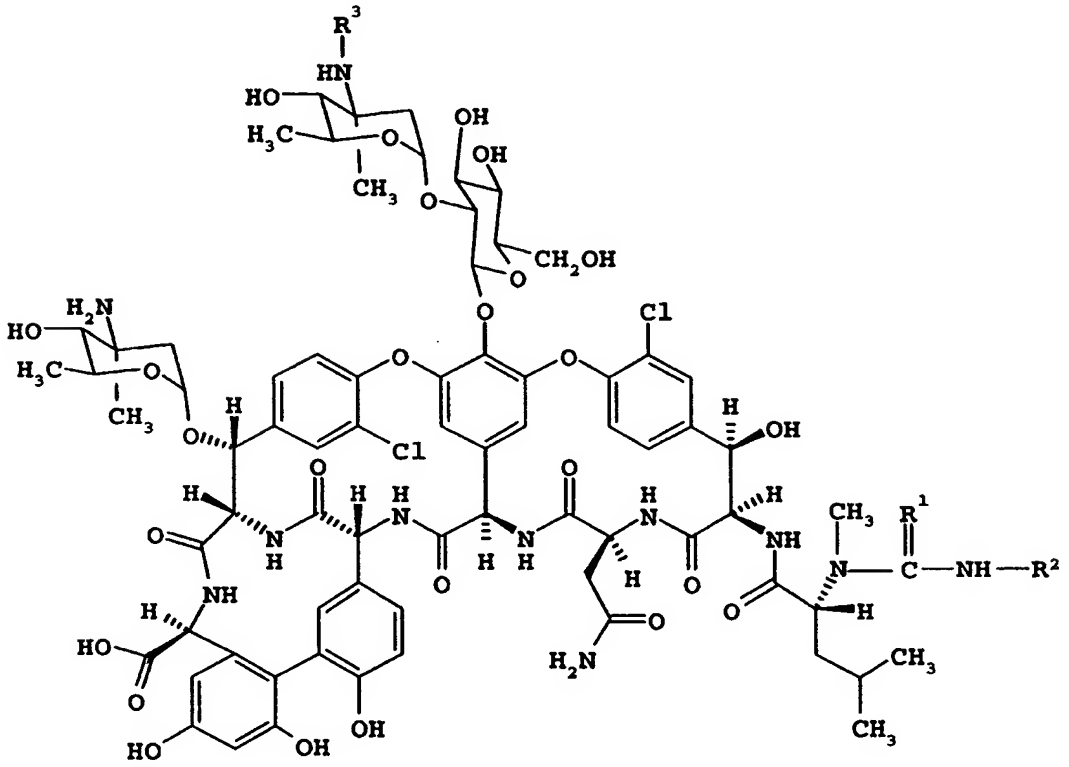
UREA AND THIOUREA DERIVATIVES OF GLYCOPEPTIDES

The present invention is directed to N^{LEU}-carbamoyl and thiocarbamoyl derivatives of A82846B and N^{DISACC} variations thereof. These derivatives are useful as antibacterials and also as starting materials from which further antibacterial compounds are prepared.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compounds of the formula



wherein R^1 represents O or S;

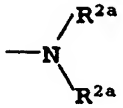
R^2 represents

alkyl of C_1-C_{10},

phenyl,

naphthyl, or

phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, benzyloxy, nitro, or



wherein each R^{2a} is independently loweralkyl of C_1-C_4; and R^3 represents hydrogen or $-CH_2-R^{3a}$ wherein R^{3a} represents

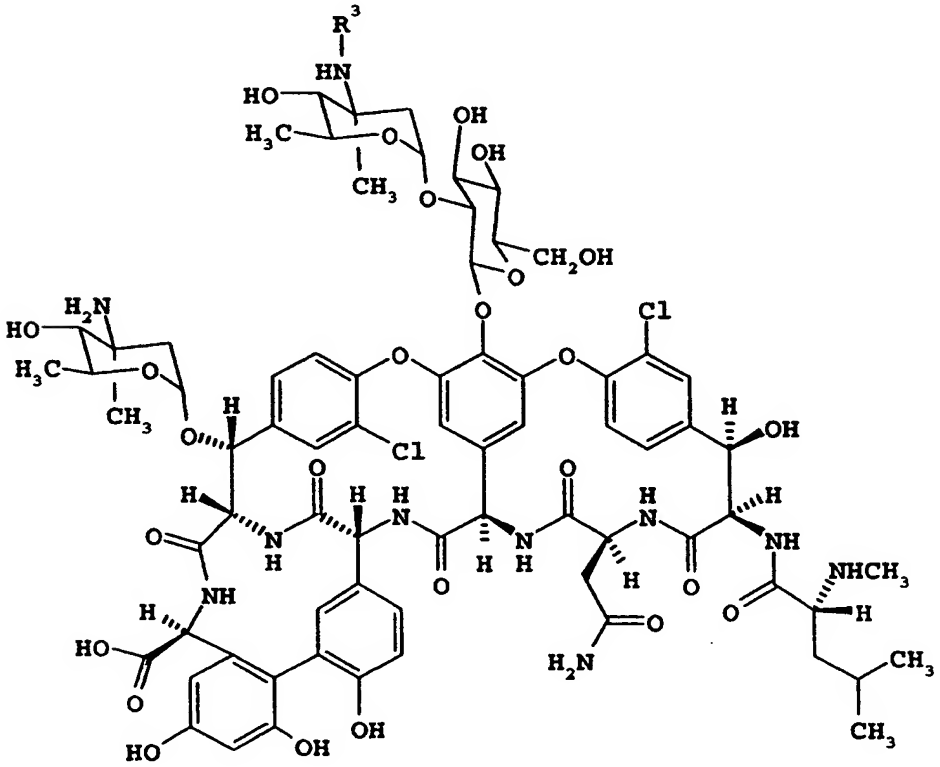
alkyl of C_1-C_{11},

alkyl of C_1-C_{11}-R^4, or

$R^4-(O_{(0\text{ or }1)}-R^4)_{0\text{ or }1}$,

wherein each R^4 is independently phenyl or phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, or loweralkylthio of C_1-C_4, and pharmaceutically acceptable salts thereof.

The compounds of the present invention are prepared by reacting a parent glycopeptide of the formula



wherein R^3 is as defined above, with an isocyanate or isothiocyanate of the formula R^1CN-R^2, wherein R^1 and R^2 are as defined above. This is the first step of the so-called Edman degradation, which is a two-step process for the cleavage of the N-terminal residue of a peptide or protein.

The reaction to prepare the present compounds is carried out in a polar solvent, such as water, in the presence of an organic base, such as pyridine. Generally the reaction is carried out at a temperature of about 15°C to about 35°C for one to five hours. The reaction is preferably carried out at a temperature from about 25°C to 30°C for one to two hours, in water with pyridine as the base. The reaction consumes equimolar amounts of the reactants but a slight excess of the isocyanate or isothiocyanate is preferred. The product is separated and purified if desired in conventional procedures. When it is desired, a salt can be prepared in standard procedures.

The following examples illustrate the preparation of the compounds of the present invention.

Preparation of Compound of Example 22

N^{DISACC}-(p-(p-Chlorophenyl)benzyl)A82846B trihydrochloride (100.0 mg, 0.0526 mmol) was dissolved in 10 ml H_2O - pyridine (1:1 v/v) and treated with phenyl isothiocyanate (0.010 ml, 0.083 mmol). The resulting mixture was stirred at room temperature for 1 hour at which time HPLC analysis indicated complete consumption of the starting material. The reaction mixture was concentrated *in vacuo* and the crude product was purified by preparative HPLC to give 76.6 mg (76% yield) of N^{LEU}-(phenylthiocarbamoyl)-

N^{DISACC}-(p-(p-chlorophenyl)benzyl)A82846B. FAB-MS: calc. For $C_{93}H_{102}Cl_3N_{11}O_{26}S$ 1925.5, obtained 1928.5 (M+3).

Preparation of Compound of Example 23

A82846B triacetate (270 mg, 0.157 mmol) was dissolved in 30 ml H_2O - pyridine (1:1 v/v) and treated with phenyl isocyanate (0.030 ml, 0.277 mmol). The resulting mixture was stirred at room temperature for 1 hour at which time HPLC analysis indicated complete consumption for the starting material. The reaction mixture was concentrated *in vacuo* and the crude product was purified by preparative HPLC to give 62.5 mg (23% yield) of N^{LEU}-(phenylcarbamoyl)-A82846B. FAB-MS: Calc. For $C_{80}H_{93}Cl_2N_{11}O_{27}$ 1709.6, obtained 1712.1 (M+3).

The HPLC procedures reported in these examples were as follows:

Analytical: Reactions were monitored by analytical HPLC using a Waters C_{18} μBondapak or Novapak C_{18} column (3.9x300 mm) and UV detection at 280 nm. Elution was accomplished with a linear gradient of 5% CH_3CN - 95% buffer to 80% CH_3CN - 20% buffer over 30 minutes. The buffer used was 0.5% triethylamine in water, adjusted to pH 3 with H_3PO_4.

Preparative: Crude reaction mixtures were purified by preparative HPLC using a Waters C_{18} Nova-Pak column (40x300 mm) and UV detection at 280 nm. Elution was accomplished with a linear gradient of 5% CH_3CN - 95% buffer to 80% CH_3CN - 20% buffer over 30 minutes. The buffer used was 0.5% triethylamine in water, adjusted to pH 3 with H_3PO_4. The

desired fractions were subsequently desalted with a Waters C_{18} Sep-Pak (35 cc) followed by lyophilization.

Compounds were desalted as follows. A Waters Sep-Pak cartridge was pre-wet with methanol (2-3 column volumes) then conditioned with water (2-3 column volumes). The sample, dissolved in a minimum volume of water, was loaded onto the Sep-Pak column which was then washed with water (2-3 column volumes) to remove the unwanted salts. The product was then eluted with an appropriate solvent system, typically 1:1 CH_3CN/H_2O, CH_3CN, and/or methanol. The organic solvent component was removed *in vacuo* and the resulting aqueous solution lyophilized to give the final product.

Representative compounds of the present invention are listed in the following table:

TABLE I

Ex #	Name	FAB-MS	M+X	Analytical HPLC, min
1	N^{LEU}-(PHENYLTHIOCARBAMOYL)A82846B	1728.5	3	18.2*
2	N^{LEU}-(PHENYLTHIOCARBAMOYL)-N^{DISACC}-(p-CHLOROBENZYL)A82846B	1852.3	3	21.4*
3	N^{LEU}-(PHENYLTHIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)-A82846B	1911.0	3	23.6*
4	N^{LEU}-(PHENYLTHIOCARBAMOYL)-N^{DISACC}-(p-PHENYLBENZYL)A82846B	1894.5	3	23.2*
5	N^{LEU}-(1-NAPHTHYLTHIOCARBAMOYL) A82846B	1778.5	3	19.8*
6	N^{LEU}-(1-NAPHTHYLTHIOCARBAMOYL)-N^{DISACC}-(p-CHLOROBENZYL)A82846B	1902.5	3	15.4*
7	N^{LEU}-(1-NAPHTHYLTHIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1960.6	3	17.1*
8	N^{LEU}-((p-CHLOROPHENYL)-THIOCARBAMOYL) A82846B	1763.0	4	20.5*
9	N^{LEU}-((p-METHOXYPHENYL)-THIOCARBAMOYL)A82846B	1757.3	2	21.0*
10	N^{LEU}-((p-CHLOROPHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1944.3	3	26.9*
11	N^{LEU}-((p-METHOXYPHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1940.3	3	26.0*
12	N^{LEU}-((p-CHLOROPHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-CHLOROBENZYL)A82846B	1887.5	4	24.8*

TABLE I (Cont.)

Ex #	Name	FAB-MS	M+X	Analytical HPLC, min
13	N^{LEU}-((p-METHOXYPHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-CHLOROBENZYL)A82846B	1882.5	3	25.2*
14	N^{LEU}-((p-NITROPHENYL)-THIOCARBAMOYL)A82846B	1774.0	3	19.1*
15	N^{LEU}-((p-(DIMETHYLAMINO)-PHENYL)THIOCARBAMOYL)A82846B	1771.4	3	17.6*
16	N^{LEU}-((p-(BENZYLOXY)PHENYL)-THIOCARBAMOYL)A82846B	1834.4	3	23.3*
17	N^{LEU}-((p-n-BUTYLPHENYL)-THIOCARBAMOYL)A82846B	1784.4	3	17.0*
18	N^{LEU}-((p-n-BUTYLPHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1966.5	3	21.4**
19	N^{LEU}-((p-(DIMETHYLAMINO)-PHENYL)THIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1953.3	3	17.1**
20	N^{LEU}-((p-(BENZYLOXY)PHENYL)-THIOCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	2016.3	3	21.1**
21	N^{LEU}-(PHENYLTHIOCARBAMOYL)-N^{DISACC}-(p-n-BUTYLBENZYL)-A82846B	1874.6	3	19.0**
22	N^{LEU}-(PHENYLTHIOCARBAMOYL)-N^{DISACC}-(p-(p-CHLOROPHENYL)-BENZYL)A82846B	1928.5	3	20.3**
23	N^{LEU}-(PHENYLCARBAMOYL)A82846B	1712.1	3	13.8**
24	N^{LEU}-(PHENYLCARBAMOYL)-N^{DISACC}-(p-PHENOXYBENZYL)A82846B	1894.2	3	18.9**
25	N^{LEU}-(n-DECYLTHIOCARBAMOYL)-A82846B	1792.4	3	N.A.

*Waters C_{18} Nova-Pak column

**Waters C_{18} μBondapak

The compounds of the present invention are useful for the treatment of bacterial infections. Therefore, in another embodiment, the present invention is directed to a method for controlling a bacterial infection in a host animal, typically a warm-blooded animal, which comprises administering to the host animal an effective, antibacterial amount of a compound of the present invention. In this embodiment, the compounds can be used to control and treat infections due to various bacteria, but especially gram-positive bacteria. In a preferred embodiment, the compounds are used to control and treat infections due to bacteria resistant to existing antibacterials. For example, certain bacteria are resistant to methicillin, and yet others are resistant to vancomycin and/or teicoplanin. The present compounds provide a technique for controlling and treating infections due to such resistant bacterial species.

In carrying out this embodiment of the invention, the compounds of the present invention can be administered by any of the conventional techniques, including the oral route and parenteral routes such as intravenous and intramuscular. The amount of compound to be employed is not critical and will vary depending on the particular compound employed, the route of administration, the severity of the infection, the interval between dosings, and other factors known to those skilled in the art. In general, a dose of from about 0.5 to about 100 mg/kg will be effective; and in many situations, lesser doses of from about 0.5 to about 50 mg/kg will be effective. A compound of the present invention can be administered in a single dose, but in the known manner of antibacterial therapy, a compound of the present invention is typically administered repeatedly over a period of time, such as a matter of days or weeks, to ensure control of the bacterial infection.

Also in accordance with known antibacterial therapy, a compound of the present invention is typically formulated for convenient delivery of the requisite dose. Therefore, in another embodiment, the present invention is directed to a pharmaceutical formulation comprising a compound of the present invention, in combination with a pharmaceutically-acceptable carrier. Such carriers are well known for both oral and parenteral routes of delivery. In general, a formulation will comprise a compound of the present invention in a concentration of from about 0.1 to about 90% by weight, and often from about 1.0 to about 3%.

The antibacterial efficacy of the present compounds is illustrated by Table II. The minimal inhibitory concentrations (MICs) were determined using a standard broth micro-dilution assay.

TABLE II. Antibacterial Activity, Minimal Inhibitory Concentration (MIC) Against Various Organisms*

Ex #	RESISTANT	SENSITIVE	SA 446	SA 489	SA 447	SH 105	SH 415	SE 270	SPY C203	SPN P1
1	>128	16	8	4	8	64	>64	8	2	2
2	128	3.4	2	2	2	8	16	4	0.125	0.25
3	16	1.7	4	2	1	2	2	1	≤.06	
4	14	4	2	0.125	1	2	1	0.5	≤0.06	0.125
5	>128	9.5	8	>64	8	64	>64	32	0.5	1
6	128	5	2	16	4	4	8	64	≤0.06	≤0.06
7	19	3	4	2	1	4	2	0.5	0.25	≤0.06
8	>128	8	2	2	4	16	64	8		
9	>128	21	8	4	8	32	32	16		
10	9.5	1.7	4	2	2	1	2	2		
11	38	2.6	4	2	2	1	2	2		
12	128	3.5	4	1	1	2	4	1	≤0.06	≤0.06
13	>128	3.5	4	2	2	4	8	2	≤0.06	≤0.06
14	>128	3.5	2	2	4	16	32	4	≤0.06	0.25
15	>128	24	8	4	16	>64	>64	16	0.25	0.25
16	>128	7	1	0.5	1	8	64	4	≤0.06	0.125
17	>128	6.1	2	1	1	4	32	2	0.25	≤0.06
18	4.7	1.7	2	2	2	2	2	2	0.25	2
19	19	2.6	2	2	2	2	4	2	0.25	2
20	9.5	5.6	4	2	2	2	2	1	≤0.06	4
21	32	2.6								
22	6.7	2.6	2	1	1	1	2	0.5	≤.06	≤.06
23	>128	5.3	4	1	4	0.5	64	4		
24	16	0.87	2	1	1	0.25	1	1		

ABBREVIATIONS	ORGANISM
RESISTANT	*Enterococcus faecium* and *faecalis* (geometric mean of 4-6 isolates)
SENSITIVE	*Enterococcus faecium* and *faecalis* (geometric mean of 4-6 isolates)
SA446	*Staphylococcus aureus* 446
SA489	*Staphylococcus aureus* 489
SA447	*Staphylococcus aureus* 447
SH 105	*Staphylococcus haemolyticus* 105
SH 415	*Staphylococcus haemolyticus* 415
SE 270	*Staphylococcus epidermidis* 270
SPY C203	*Streptococcus pyogenes* C203
SPN P1	*Streptococcus pneumoniae* P1

The N^{LEU}-thiocarbamoyl compounds of the present invention can also be employed as starting materials to other antibacterial compounds. This use is illustrated by the following reaction sequence:

present compounds ⟶ hexapeptides ⟶ N^1-alkylated hexapeptides

Thus, a present compound is treated with an organic acid, preferably trifluoroacetic acid, in a non-polar solvent, and at a temperature of from about 0°C to 35°C. This treatment, the second step of an Edman degradation, results in the loss of the leucine group including the thiocarbamoyl substituent. The resulting "hexapeptides" exhibit antibacterial activity and can be employed as described above for the present compounds.

The hexapeptide can thereafter be reductively alkylated to introduce an alkyl group on the amine freed up by the preceding process, the "N^1" amine. Alkylation is achieved by reacting the hexapeptide with an aldehyde to form a Schiff's base, which is then reduced to obtain the N^1-alkylhexapeptide. Both reactions are carried out in a polar

solvent, such as DMF, and at temperatures of 0-100°C, preferably 60-70°C. The preferred reducing agent is sodium cyanoborohydride. In one embodiment, the reducing agent is added at the same time as the hexapeptide and aldehyde. The resulting N^1-alkylated hexapeptides are useful as antibacterials and can be employed as described above for compounds of the present invention.

wherein each R^{2a} is independently loweralkyl of C_1-C_4; and R^3 represents hydrogen or $-CH_2-R^{3a}$ wherein R^{3a} represents

alkyl of C_1-C_{11},

alkyl of C_1-C_{11}-R^4, or

$R^4-(O_{(0\text{ or }1)}-R^4)_{0\text{ or }1}$,

wherein each R^4 is independently phenyl or phenyl substituted by one or two substituents, each of which is independently halo, loweralkyl of C_1-C_4, loweralkoxy of C_1-C_4, or loweralkylthio of C_1-C_4, or a pharmaceutically acceptable salt thereof.

2. A compound of Claim 1 in which R^1 is S.

3. A compound of Claim 1 in which R^2 is phenyl.

4. A compound of Claim 1 in which R^3 is $-CH_2-R^{3a}$.

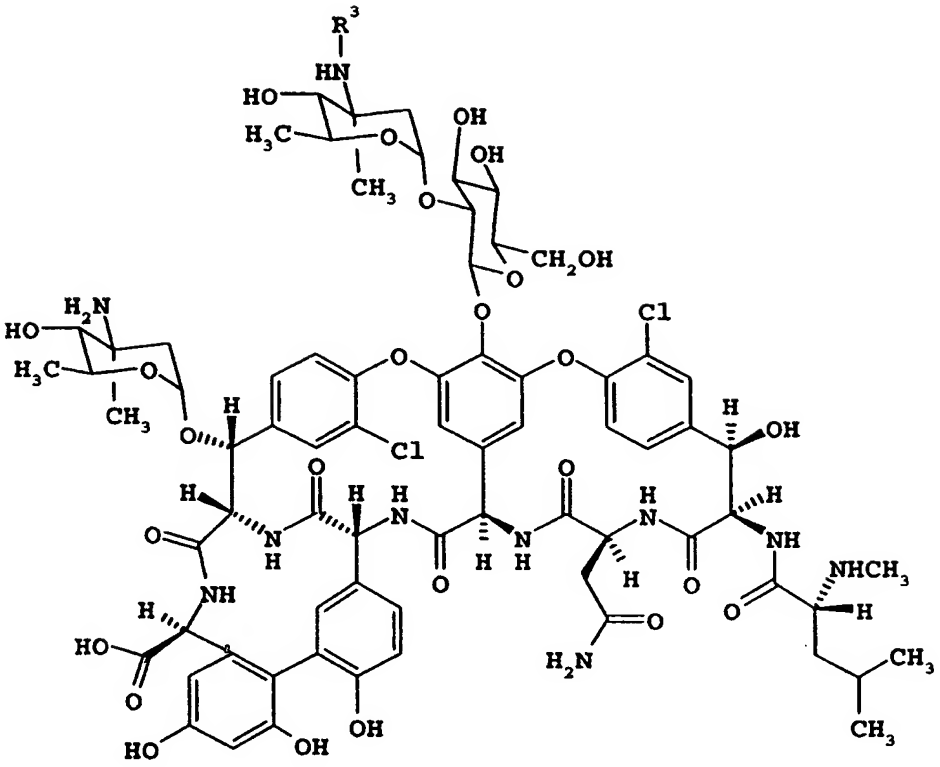
5. A compound of Claim 1 in which R^3 is p-(p-chlorophenyl)benzyl.

6. A pharmaceutical formulation comprising a compound of Claim 1 in combination with a pharmaceutically-acceptable diluent or carrier.

7. A method of treating a bacterial infection in a host comprising the step of administering to the host an effective amount of a formulation of Claim 6.

8. A method of Claim 7 wherein the bacterial infection is attributable to a vancomycin-resistant-enterococcus.

9. A process for the preparation of a compound as claimed in Claim 1 which comprises reacting a parent glycopeptide of the formula



wherein R^3 is as defined in Claim 1, with an isocyanate or isothiocyanate of the formula R^1CN-R^2 wherein R^1 and R^2 are as defined in Claim 1, and if desired thereafter forming a pharmaceutically acceptable salt.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/09131

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00; C07K 9/00.
US CL :514/2, 8, 9, 11; 530/317, 322.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 9, 11; 530/317, 322.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, BIOSIS, MEDLINE, MARPAT.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,534,420 A (DEBONO et al.) 09 July 1996, see entire document.	1-9
Y	WO 96/30401 A1 (ELI LILLY AND COMPANY) 03 October 1996, see entire document.	1-9
Y	EP 0 667 353 A1 (ELI LILLY AND COMPANY) 16 August 1995, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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